AD_____

Award Number: W81XWH-10-1-0872

TITLE: Inhibition of Orthopaedic Implant Infections by Immunomodulatory Effects of Host Defense Peptides

PRINCIPAL INVESTIGATOR: Edward Greenfield, PhD

CONTRACTING ORGANIZATION: Case Western Reserve University
Cleveland OH 44106-1712

REPORT DATE: October 2012

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2012	Annual	15 September 2011- 14 September 2012

4. TITLE AND SUBTITLE
Inhibition of Orthopaedic Implant Infections by Immunomodulatory Effects of Host Defense Peptides

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0872

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Edward Greenfield, PhD

E-Mail: emg3@cwru.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Case Western Reserve University
Cleveland OH 44106-1712

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT- Host defense peptides represent a promising new approach to inhibit infection. The anti-infective actions of these peptides are primarily due to their immunomodulatory effects. Since they regulate multiple aspects of the mammalian immune system, host defense peptides are also less likely to induce bacterial resistance than are traditional antibiotics. The local delivery of anti-bacterial agents allows for both high local concentrations to increase efficacy and low systemic levels to reduce toxicity. A promising strategy for the local delivery of anti-bacterial agents is to bind them directly to the surfaces of orthopaedic implants. The progress in the second year of this project has demonstrated:
1. Development of a quantitative and reproducible murine model of orthopaedic implant infection.
2. Preliminary results indicate that the soluble host defense peptide reduces orthopaedic implant infection in the murine model.
3. Preliminary results indicate that the soluble host defense peptides increases osseointegration in mice that were not inoculated with bacteria.
The host defense peptides have the potential to substantially reduce infections of fractures sustained on the battlefield and in civilian settings. If the synthetic peptide reduces infections in the studies proposed in this application, more extensive pre-clinical testing would precisely determine its potential benefits and risks and determine whether the peptide is a high priority for human trials.

15. SUBJECT TERMS- Orthopaedic infections, Host Defense Peptides, Murine model, Staphylococcus aureus, Acinetobacter baumannii

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	7	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

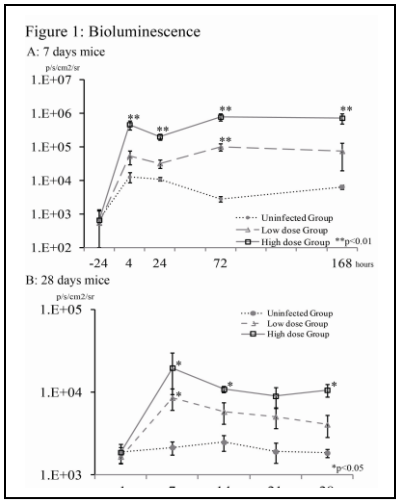
INTRODUCTION:

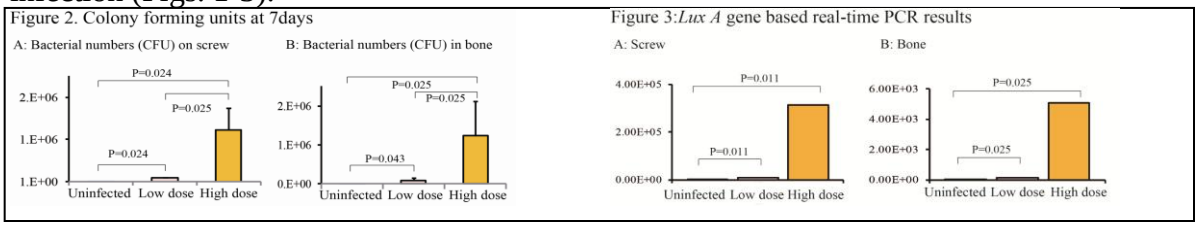
Host defense peptides represent a promising new approach to inhibit infection. The anti-infective actions of these peptides are primarily due to their immunomodulatory effects. Since they regulate multiple aspects of the mammalian immune system, host defense peptides are also less likely to induce bacterial resistance than are traditional antibiotics. The local delivery of anti-bacterial agents allows for both high local concentrations to increase efficacy and low systemic levels to reduce toxicity. A promising strategy for the local delivery of anti-bacterial agents is to bind them directly to the surfaces of orthopaedic implants. Our hypotheses are:

1. Soluble host defense peptides reduce infection of orthopaedic implants.
2. Host defense peptides bound to orthopaedic implant surfaces reduce infection.

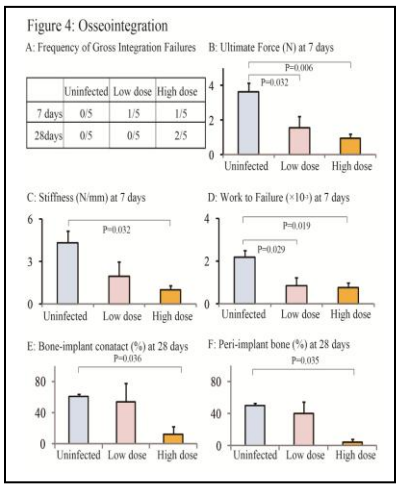
BODY:

One of the primary areas of progress in this year has been the development of the murine model of orthopaedic implant infection. Appropriate concentrations of Staph. aureus were identified that reproducibly provide chronic implant infection without causing any signs of systemic sepsis. In mice without bacteria, BLI was low at all time points (Fig. 1) and no bacteria were detectable either adherent to the implants or in the bones by counting colony-forming units (Fig. 2) or by quantitative PCR of a marker gene (Fig. 3). Importantly, bacterial inoculation dose-dependently increased all of those measures of infection (Figs. 1-3).

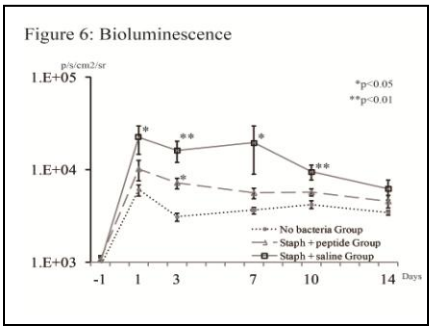


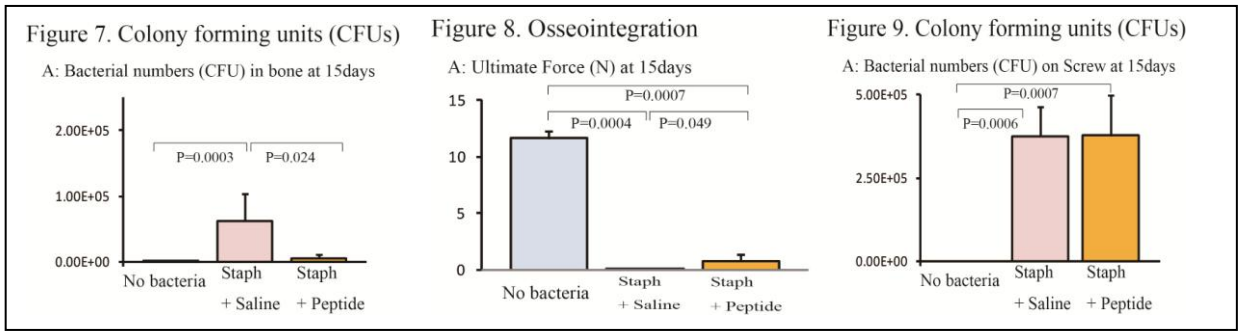


The long-term ability to detect BLI is likely due to the use of *S. aureus* with the *lux* operon in a stable plasmid as shown by other investigators [1]. Osseointegration was significantly decreased in the groups with the higher dose of bacteria. Thus, there was a higher rate of gross integration failure (Fig. 4A) and reduced biomechanical (Figs. 4B-D) and histomorphometric (Fig. 4E-F) measures of osseointegration. Representative histological images of the impaired osseointegration are shown in Figure 5. Taken together the results described in this paragraph demonstrate that the higher bacterial inoculation dose provides a quantitative and reproducible murine model of orthopaedic implant infection that includes impaired osseointegration.

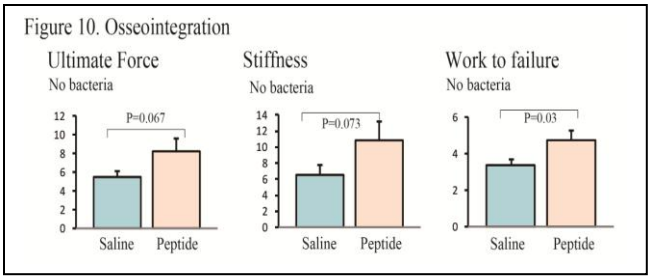


With the model described in the previous paragraph, we are beginning to test hypothesis #1 that soluble host defense peptides reduce infection of orthopaedic implants. Preliminary results indicate that three daily injections of the soluble host defense peptide reduces BLI (Fig. 6) and CFUs in the bone (Fig. 7) and modestly increases biomechanical measures of osseointegration (Fig. 8). Interestingly, there was no effect on CFUs adherent to the implant (Fig. 9).





Preliminary results also indicate that the soluble host defense peptide increases osseointegration in mice that were not inoculated with bacteria (Fig. 10). Since osseointegration is impaired by inflammation, this result may be due to the ability of the host defense peptide to reduce inflammation [2-3].



We are currently confirming the results described in this paragraph in additional mice and determining whether the effects of the soluble host defense peptide can be increased, for example, by increasing the number of injections of the peptide.

KEY RESEARCH ACCOMPLISHMENTS:

1. Development of a quantitative and reproducible murine model of orthopaedic implant infection (Figs. 1-5).

2. Preliminary results indicate that the soluble host defense peptide reduces orthopaedic implant infection in the murine model (Figs. 6-9).

3. Preliminary results indicate that the soluble host defense peptides increases osseointegration in mice that were not inoculated with bacteria (Fig. 10).

REPORTABLE OUTCOMES:

Development of the murine model was reported in a poster presented at the Military Health System Research Symposium, Ft Lauderdale, Florida, 13-16 August 2012 (see Appendix)

CONCLUSION:

The host defense peptides have the potential to substantially reduce infections of fractures sustained on the battlefield and in civilian settings. If the synthetic peptide reduces infections in the studies proposed in this application, more extensive pre-clinical testing would precisely determine its potential benefits and risks and determine whether the peptide is a high priority for human trials.

REFERENCES:

[1] Bernthal et al, 2010 PLoS ONE 5:e12580
[2] Wieczorek et al Chem Biol. 2010 17:970-80
[4] Achtman et al, Sci Trans Med 2012 4:135ra64

APPENDIX:

Poster presented at the Military Health System Research Symposium, Ft Lauderdale, Florida, 13-16 August 2012

Murine Model of Impaired Osseointegration due to Implant Infection

Hyonmin Choe, Joscelyn Tatro, David Corn, Steve Marshall, Lindsay A Bonsignore, Zhenghong Lee, Robert A Bonomo, and Edward M Greenfield
Case Western Reserve University and Louis Stokes Cleveland VA Medical Center
Cleveland, OH

INTRODUCTION

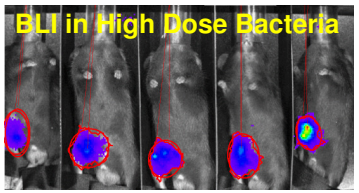
Infection is the most difficult complication of orthopaedic implant surgery and often impairs osseointegration of the implant. Treatment typically requires two-stage revisions and extended intravenous antibiotic therapy. The increasing prevalence of multidrug-resistant (MDR) bacteria has made antibiotic treatment especially difficult. Development of novel therapeutic agents is therefore required. Murine models are extremely useful for this purpose due to the availability of transgenic and knockout strains. Moreover, the reduced cost of mice compared to larger animals facilitates screening of novel therapeutic strategies.

A murine infection model that assesses implant osseointegration has not yet been established. In this study, we developed murine model of osseointegration to compare sterile implants and implants with adherent bacteria based on our previously validated murine model of osseointegration [1,2]. We quantified the bacterial burden by bioluminescence imaging (BLI) and counting of colony forming units (CFUs) and measured osseointegration by histomorphometry and biomechanical pull-out testing.

METHODS

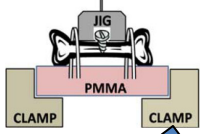
1.0 ×10^5 or 3×10^6 CFUs/implant of bioluminescent *Staph. aureus* (Xen36 strain, Caliper Life Sciences) were adhered to rigorously cleaned titanium alloy screw shaped implants (1 mm diameter, 3.2 mm length) immediately before insertion into pilot holes in the mid-diaphysis of the femur of 6-7 week old male C57BL/6 mice [1-2]. BLI was measured 24 hours before surgery as a baseline and at the indicated time-points after implant placement using a Xenogen IVIS 200 system (Caliper Life Sciences). BLI results are reported as photons/second/cm^2/steradian in the region of interest with subtraction of the background signal. Mice were euthanized 7 or 28 days after implant placement and osseointegration was measured by histomorphometry and biomechanical pull-out testing [1,2]. After pull-out testing, implants were sonicated and femurs were homogenized and bacterial CFUs were determined in the sonicates and the homogenates [3].

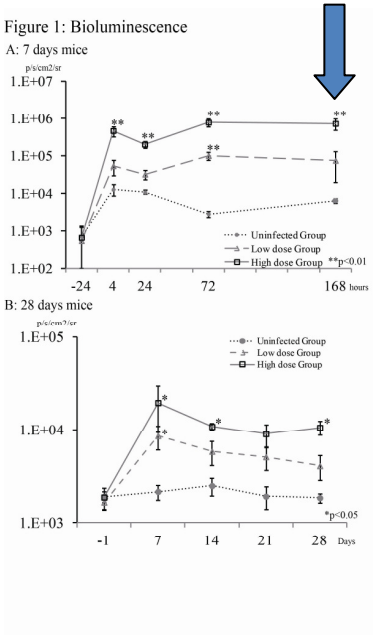
RESULTS

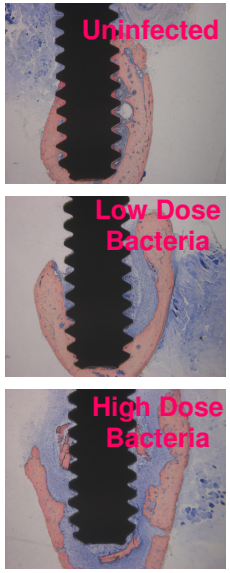


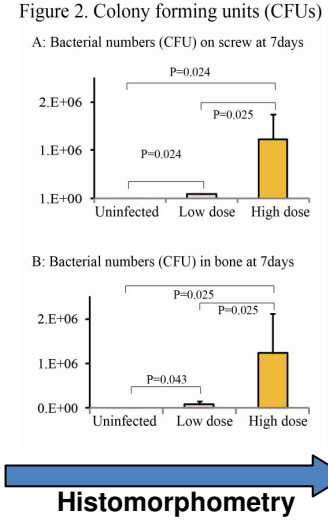
Dissected Femur

Biomechanical analysis

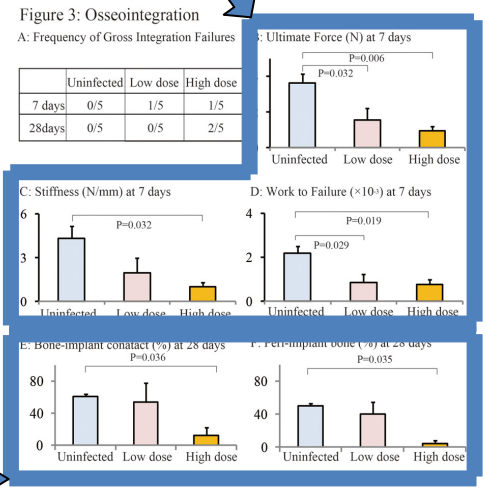








Histomorphometry



DISCUSSION

1. Signs of systemic sepsis were not observed in any of the mice and the BLI imaging demonstrated that the infection was localized to the implant site.
1. In mice without bacteria, BLI was low at all time points (Fig 1).
2. Bacterial inoculation increased both BLI (Fig 1) and CFUs on the implants and in the bones (Fig 2).
3. Osseointegration was significantly decreased in the groups with the higher dose of bacteria (Fig 3).
4. The higher bacterial inoculation dose provides a quantitative and reproducible murine model of orthopaedic implant infection that includes impaired osseointegration.
5. The long-term ability to detect BLI is likely due to the use of *S. aureus* with the *lux* operon in a stable plasmid as shown by other investigators [3].
6. With this model, we are now in a position to assess novel methods of preventing/treating infections.

REFERENCES

[1] Bonsignore et al, 2011, Bone 49:923-30
[2] Bonsignore et al, 2012, submitted
[3] Bernthal et al, 2010 PLoS ONE 5:e12580

ACKNOWLEDGEMENT

USAMRAA
Peer Reviewed Orthopaedic Research Program
Idea Development Award